

UNIVERSITE DE GENEVE
Département de médecine

FACULTE DE MEDECINE
Professeur A.F. MULLER
Docteur H. FAVRE, PD

**BASAL ACTIVITY OF THE NATRIURETIC
FACTOR EXTRACTED FROM THE RAT KIDNEY
AS A FUNCTION OF THE DIET
AND ITS ROLE IN THE REGULATION
OF THE ACUTE SODIUM BALANCE**

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présentée à la faculté de médecine de l'Université de Genève
pour obtenir le grade de docteur en médecine

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François LOUIS

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Thèse No 3720

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La Faculté de médecine, sur le préavis du professeur A.F. Muller et docteur H. Favre Privat-Docent, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

Genève, le 25 janvier 1979

Le doyen :
André Cruchaud

Thèse No 3720



Je tiens à exprimer toute ma reconnaissance au professeur A.F. Muller et au docteur H. Favre qui ont bien voulu accepter de diriger ce travail.

La réalisation de cette thèse a bénéficié de la collaboration de Mesdemoiselles M. Gourjon, G. Lucot et E. Gaillard, laborantines, de Madame M.C. Seydoux, secrétaire. Qu'elles trouvent ici l'expression de ma profonde gratitude.

ABSTRACT. The urine of normal dogs and human submitted to a chronic high salt intake with or without fludrocortisone contains an inhibitor of the sodium transport that produces a natriuresis in the rat. These studies represent an effort to detect the same inhibitor in the renal tissue of the rat after acute saline expansion up to 10% of the body weight. Observations were made under basal conditions in rats maintained on a sodium intake of 0, 3, 6 mEq/day and repeated under acute saline expansion. The same Sephadex G-25 gel filtration fraction containing the inhibitor previously described was tested using a rat bioassay. Under basal conditions only a small natriuretic activity of the fraction is detectable in rats given 6 mEq/sodium a day. However, there is a good correlation between the natriuretic activity of the fraction found in the kidney and the degree of sodium balance and sodium excretion in the rat.

In contrast, the fraction extracted from the kidneys after acute expansion induces a significant natriuresis in the bioassay, the higher effect being observed with the fractions from rats on a previous 3 and 6 mEq/sodium intake which also respond by a larger natriuresis than those maintained on a 0 mEq/sodium diet ($p < 0.01$). The present studies demonstrate a relationship between the basal activity of the natriuretic factor and the sodium content of the diet. There is also a relationship between the natriuretic response to an acute load of sodium which is modulated by the previous diet and the degree of activity of the natriuretic fraction. Thus, natriuretic factor also correlates with the degree of acute sodium balance.

INTRODUCTION

While evidences have been established beyond doubt that factors other than aldosterone and the filtered sodium load are involved in the regulation of the sodium excretion (1-3), searches from different laboratories support the view that an unknown substance possessing a natriuretic activity may play a role in the control of the sodium balance. Most of the studies have been performed in uremic (4-6) and normal (7-10) animals and human subjected to a chronic expansion of the extracellular fluid volume. Such a natriuretic hormone has been consistently found in the urine of dogs and human in relation to the need of excreting large amount of sodium by the kidneys (7, 8). The majority of the authors have fractionated the urine through a G-25 Sephadex column and then have defined the natriuretic hormone as a substance of a low molecular weight which increases the sodium excretion in absolute and fractional terms when injected into a rat used as a bioassay. However, it was to be noted that some authors have found alone or in addition to this low molecular weight material an other substance of high molecular weight which seems to promote, when injected into the rat, a natriuretic response characterized by a different profil than the one observed when the low molecular weight substance is tested (11). The relationship between the two substances remains to be elucidated. A detailed review of the subject has recently appeared in the literature (12).

Our previous personal efforts have been directed to models of dogs and human with renal functions maintained on high salt intakes and given or not mineralo-corticoid hormone (7, 8). In both species an inhibitor of the sodium transport of low molecular weight was demonstrated from the urine extracts in correlation with the degree of sodium excretion and retention. If such a natriuretic factor plays a determinant physiological role in the regulation of the chronical homeostasis of the extracellular fluid, it should be at work in response to an acute expansion of this milieu.

As it is known that natriuretic factor could be derived from the renal tissue of the rat (13), although the relationship between the acute sodium balance and the degree of activity of the natriuretic factor in the same animal was not demonstrated, natriuretic factor was extracted from the kidney tissue and prepared according to the previous methodology used in the isolation of the natriuretic fraction from urine of dogs and human.

Therefore, the present experiments were undertaken to determine whether or not the changes in the level of the activity of the natriuretic factor found in the rat kidney and following an acute saline expansion, correlate with the situations of the acute sodium balance. In addition, basal natriuretic activity of the factor was investigated under different basal conditions.

The results demonstrate that an inhibitor of the sodium transport is consistently detectable in the kidney tissue of the rats following an acute saline expansion. The activity of this inhibitor is greater in the rats previously fed a high salt diet than in those submitted to a depleted sodium diet. In addition, a good correlation exists between the degree of activity of the natriuretic factor and the requirement for the maintenance of the sodium balance in all the situations considered.

METHODS

Experiments were performed on 37 normal Wistar rats (Physiopathology Institute Bern) weighing between 300 and 350 g. These animals were separated in two series of 3 groups each referred as groups A, B, C. - first series, and A_E , B_E , and C_E - second series. Then the basic protocol was identical for all the groups. Rats were confined in an individual metabolic cage for a ten-day period during which they received 25 g of a synthetic sodium deficient food containing 2.2 mEq sodium per 100 g of dry powder (C 1036 Altromin D 4937 Lage (Lipe)).

TABLE I

Dietary Regimens and Saline Expansions of Experimental Animals

Experimental group	Number of rats	Sodium intake mEq/24 hrs.	Saline expansion
A	10	0	-
A _E	5	0	+
B	7	3	-
B _E	6	3	+
C	5	6	-
C _E	4	6	+

Rats of groups A_E, B_E and C_E were expanded over 60 minutes with an isotonic saline solution up to ten per cent of their body weight. Rats of groups A, B, and C were perfused with a sustaining glucose solution.

To these basic diets, 0, 3, 6 mEq sodium chloride a day were added to the drinking water. Thus, 3 different paired groups were investigated. Groups A and A_E were allowed to drink 25 ml of bidistilled water, groups B and B_E 25 ml of fluid containing 3 mEq sodium (1.5 ml NaCl 11.7% ad 25 ml of bidistilled water), groups C and C_E 25 ml of fluid containing 6 mEq sodium (3 ml NaCl 11.7% ad 25 ml bidistilled water). Special attention was paid to the amount of drinking solution ingested and rats which were unable to drink the total amount of fluid required were rejected.

For the 5 last days in metabolic cage, cumulative sodium balance was measured. The tenth day endogeneous creatinine and 24-hour urine collection were measured in order to determine the GFR and the FENa in 15 rats - 5 rats from each group A, B, C.

On the eleventh day, clearance studies were performed under basal conditions - groups A, B, C - or after acute saline expansion, groups A_E, B_E, C_E.

18 hours prior to these studies the dead urinary space was diminished by ligation of the dome of the bladder and then the rats were kept fasting.

The day of the experiment a polyethylene catheter (PE 25, Portex, England), was inserted into the jugular vein under a light ether anaesthesia. Then, rats were confined into a restrictive cage, and after they recovered from the surgery a priming dose of 0.7 ml of a 2.5% inulin solution (Laevosan Ag. Zurich) was injected i.v. Then, a solution of 0.25% inulin dissolved in glucose 2.5% was perfused by means of a serynge pump (model 355 SAGE Instruments, Division of Orion Research Incorporated) at a delivery rate of 0.15 ml/min. After a 60-minute equilibration, three 20-minute clearance periods were collected and blood samples were drawn in the middle of each period from the tip of the tail for measurement of sodium and inulin. At the end of the last clearance period kidneys were immediately removed under ether anaesthesia, and placed in 20 ml of bidistilled water adjusted to a pH of 3.4 with acetic acid. The solution was boiled for 20 min at 65⁰, then kidneys were

homogenized and centrifugated at 3.000 rpm for 25 min at 4°C. The supernate was applied into a 2.5 x 95 cm column packed with Sephadex G-25 (Pharmacia p.d.f AG, Zurich) and eluted at 4°C with a 0.01 M ammonium acetate solution adjusted to a pH of 6.8 using a Perpex pump delivering 1.2 ml solution per min (5). The effluent solution (10 tubes of 12 ml each) which appeared immediately after the salt peak and which previously has been shown to contain the inhibitor of sodium transport in the urine from patients with chronic uremia (4, 6) and in urine of normal dogs (7) was collected, lyophilized, reconstituted in 1.5 ml of bidistilled water, and stored at -25°C until the day where the activity of the fraction was tested using a bioassay. Rats of the second series (groups A_E, B_E, C_E) underwent the same preparation as described for series one, but the day of the experiment they were expanded during 60 min with an isotonic saline solution containing 0.25% inulin at a perfusion rate of 0.5 ml/min.

After a 10% expansion of the body weight was reached, saline perfusion was maintained at a 0.1 ml/min rate during which three 20-minute clearance periods were collected. Then, the kidneys were removed and proceeded as previously described for series one.

The activity of the fraction extracted from the total kidney tissue of individual rats was tested in a rat assay preparation. The fraction was adjusted to a pH of 7.35 with NaOH 0.1 N and to sodium and potassium concentrations of 140 and 4 mEq/l. The assay was performed by using normal Wistar rats weighing between 100 and 120 g. The preceding day of the experiment the dead urinary space was reduced by ligation of the dome of the urinary bladder. Animals were ether anaesthetized and a PE 25 was introduced into the jugular vein. By the end of the surgery, rats were confined in a restrictive cage. 60 minutes after they woke up, rats were perfused with a priming dose containing 0.25 ml inulin 10%, 0.2 ml NaCl 11.7% and 20 ml glucose 2.5%, over 20 minutes by means of a serynge pump at a rate of 0.4 ml/min. Then, a sustaining solution (1.25 ml inulin 10%, 0.5 ml NaCl 11.7%, 50 ml glucose 2.5%) was perfused at a rate of 0.15 ml/min. After 25 minutes of equilibration, four 10-minute clearance periods (control period) were collected, and a sample of

blood was drawn from the tip of the tail in the second control period. Then, 1 ml of the prepared fraction extracted from kidney tissue was slowly injected i.v. (10 min). Urines from this period and the ten following minutes were thrown away. The experimental period consisted of six 10-minute clearance periods. The first experimental clearance period began 10 minutes after the end of the injection of the fraction. Blood samples were taken on the second and the fourth experimental clearance period. The results were expressed as changes in per cent between the average of FENa and UNaV observed during the four control periods and the mean of the same parameters obtained during the six experimental periods according the formula :

$$FENa_i = (FENa_a - FENa_b / FENa_b) \times 100 \quad *$$

Inulin was determined by the colorimetric method of Zender (14). Creatinine was measured by the conventional method of Jaffé.

Sodium was measured by a flame spectrophotometer (model IL 543, Instrumentation Laboratory Milano), the pH by a pH meter with a combined glass electrode (pH M 62 standard pH meter, Radiometer, Copenhagen). The titration of the solution and the fractions were made with a titrator (TTT 60 Titrator, Radiometer, Copenhagen). Calculations were made with a Texas Instrument (Ti Programable 59). The variation for sets of measurements was expressed as the standard error of the mean. Statistical analyses were performed with Student's t test (paired "t" test and impaired "t" test). Significance in the text was expressed as the P value.

RESULTS

Data for cumulative sodium balance for 5-day period and physiological results on the tenth day are presented in Table II. All rats given a 0 mEq Na/day diet (group A) were in negative sodium balance averaging a mean of -0.937 ± 0.11 mEq of sodium per 5 days. Rats receiving 3 mEq Na/day

* FENa_a = FENa after the injection
FENa_b = FENa before the injection.

TABLE II
Physiological Data obtained in Metabolic Cage during
the tenth Day and 5-Day cumulative Sodium Balance

PHYSIOLOGICAL RESULTS				5-Day
RATS	G F R	U Na V	F E Na	Cumulative Na
No	ml/min	μEq/min	%	Balance Data
mEq				
GROUP A				
1	0.856	0.06	0.05	-0.932
2	0.707	0.036	0.036	-1.32
3	0.983	0.047	0.032	-0.63
4	0.537	0.13	0.16	-0.954
5	0.853	0.029	0.024	-0.851
mean	0.79	0.06	0.06	-0.937
± SE	0.07	0.017	0.02	0.11
GROUP B				
1	0.94	1.54	1.28	3.432
2	1.10	2.33	1.6	8.5
3	0.89	2.64	2.2	8.6
4	1.05	1.63	1.20	8.4
5	0.89	0.75	0.53	3.94
mean	0.974	1.77	1.36	6.57
± SE	0.043	0.33	0.27	1.18
GROUP C				
1	0.93	4.14	3.3	20.72
2	1.063	3.62	2.37	12.528
3	1.01	3.98	2.81	12.55
4	0.86	3.16	2.56	16.09
5	1.1	3.93	2.59	16.26
mean	0.99	3.76	2.72	15.63
± SE	0.042	0.17	0.16	1.51

Sodium balance is expressed on the difference between oral intake and urinary excretion without adjustment for extrarenal losses. GFR and sodium excretion were measured the tenth day with urine samples of a 24-hour period. Groups of rats are defined in Table I.

(15 mEq for 5 days - group B) retained an average of 6.57 ± 1.18 mEq of sodium, value significantly greater than the one observed in group A (B vs A $p < 0.0005$). Animals given a 6 mEq Na/day diet (30 mEq Na for 5 days - group C) had a cumulative sodium balance of 15.63 ± 1.51 mEq of Na, a result significantly higher than in group B (C vs B $p < 0.025$) and in group A (C vs A $p < 0.0005$).

The total excretion of sodium during this 5-day period was 0.937 ± 0.11 mEq of sodium/5 days for group A, 8.425 ± 2.64 mEq of sodium/5 d. for group B, and 14.37 ± 3.37 mEq of sodium/5 d. for group C.

Physiological data measured the tenth day with a 24-hour urine collection showed a GFR mean of 0.79 ± 0.07 ml/min for group A, 0.97 ± 0.043 ml/min for group B, and 0.99 ± 0.042 ml/min for group C. Mean of UNaV and FENa gave 0.06 ± 0.017 μ Eq/min and $0.06 \pm 0.02\%$ for group A, 1.77 ± 0.33 μ Eq/min and $1.36 \pm 0.27\%$ for group B, 3.76 ± 0.17 μ Eq/min and $2.72 \pm 0.16\%$ for group C. FENa and UNaV data of group B had significant higher values than in group A ($p < 0.005$ for both parameters), and the same parameters of group C were greater than those observed in group B ($p < 0.01$ and $p < 0.05$ for FENa and UNaV respectively), and than in group A ($p < 0.0005$ for both parameters).

Results of the clearance studies are given in Table III. UNaV was low for the three non-expanded groups : 0.253 ± 0.06 μ Eq/min in group A, 0.54 ± 0.32 μ Eq/min in group B, and 0.35 ± 0.15 μ Eq/min in group C. FENa respectively averaged $0.076 \pm 0.018\%$, $0.21 \pm 0.13\%$, and $0.08 \pm 0.035\%$. No statistical differences were observed between the three groups. GFR means were 2.34 ± 0.19 ml/min in group A, 2 ± 0.18 ml/min in group B, 2.97 ± 0.34 ml/min in group C.

After the rats were expanded, GFR averaged 3.16 ± 0.33 ml/min for group A_E, 2.35 ± 0.43 ml/min for group B_E, and 3.11 ± 0.57 ml/min for group C_E. For group A_E UNaV and FENa means were respectively 21.4 ± 1.88 μ Eq/min, and $5.04 \pm 0.26\%$, for group B_E 17 ± 0.9 μ Eq/min and $5.97 \pm 0.6\%$,

TABLE III
GFR and Sodium Excretion in Experimental Rats

RAT No.	GROUP A			GROUP B			GROUP C			GROUP A _E			GROUP B _E			GROUP C _E		
	GFR ml/min	UNaV μEq/min	FENa %	GFR ml/min	UNaV μEq/min	FENa %	GFR ml/min	UNaV μEq/min	FENa %	GFR ml/min	UNaV μEq/min	FENa %	GFR ml/min	UNaV μEq/min	FENa %	GFR ml/min	UNaV μEq/min	FENa %
1	2.16	0.107	0.037	2.14	2.44	0.97	3.407	0.351	0.075	2.44	16.27	4.9	1.92	16.405	7.23	3.38	31.1	6.56
2	2.063	0.179	0.061	1.83	0.62	0.28	4.0	0.244	0.041	2.94	18.5	4.77	1.97	20.73	7.64	3.076	23.6	5.33
3	2.31	0.056	0.021	1.01	0.045	0.038	2.57	0.12	0.035	4.31	25.32	4.28	1.69	13.5	6.0	4.39	41.8	6.4
4	3.12	0.492	0.111	2.42	0.058	0.017	1.98	0.087	0.024	3.45	26.0	5.75	4.49	22.7	4.03	1.63	15.76	6.93
5	1.26	0.008	0.005	2.24	0.37	0.28	2.9	0.96	0.22	2.66	21.1	5.57	2.21	18.5	6.52	-	-	-
6	2.762	0.321	0.008	2.168	0.101	0.036	-	-	-	-	-	-	1.73	10.14	4.43	-	-	-
7	2.185	0.301	0.1	2.26	0.15	0.046	-	-	-	-	-	-	-	-	-	-	-	-
8	2.21	0.09	0.038	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	3.46	0.414	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1.94	0.563	0.206	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mean	2.34	0.253	0.076	2.0	0.54	0.21	2.97	0.35	0.08	3.16	21.4	5.04	2.35	17.0	5.97	3.11	28.0	6.3
± SE	0.19	0.06	0.018	0.18	0.32	0.13	0.34	0.15	0.035	0.33	1.88	0.26	0.43	0.9	0.6	0.57	5.5	0.32

GFR, UNaV and FENa were determinate after the animals were perfused with a sustaining glucose solution during one hour for groups A, B, C, and after the rats were expanded with a saline solution for groups A_E, B_E, C_E. Groups of rats are defined in Table I.

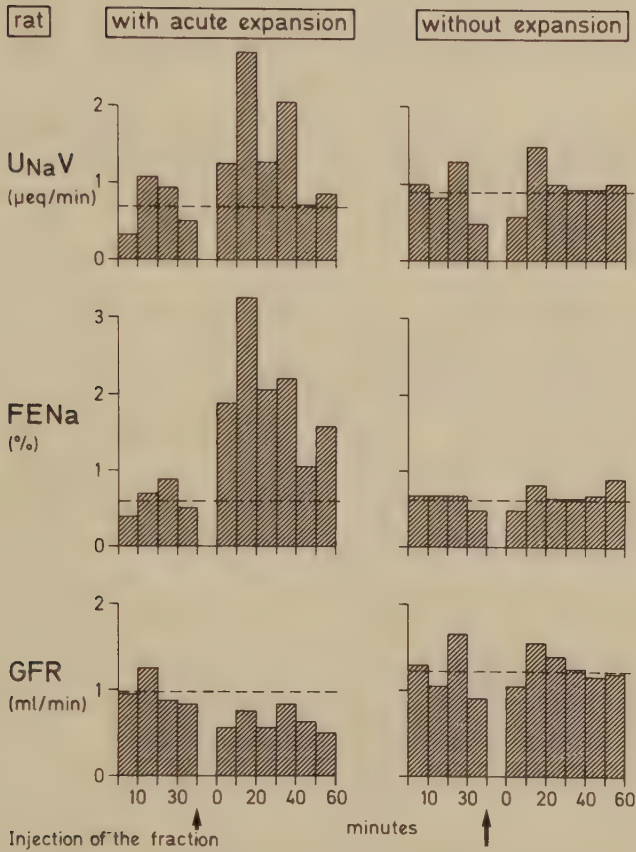


FIGURE I. Effects observed with an active fraction obtained from total renal tissue of an expanded rat (left panel) and a non-expanded rat (right panel) on U_{Na}V, FENa and GFR in the assay. The broken line represents the mean base line control value observed before infusion of the fraction.

for group C_E $28 \pm 5.5 \mu\text{Eq/min}$ and $6.3 \pm 0.32\%$. FENa was significantly greater in the groups receiving a basal salt intake - B_E and C_E - than in the depleted group (A_E) : $p < 0.05$

Fig. 1 depicts the effects in the rat of two fractions derived from the kidney tissue obtained from a rat belonging to an expanded group (B_E), and from a rat of an non-expanded group (C). Values are shown for UNaV, FENa and GFR.

Using fraction from non-expanded rat UNaV increased from $0.907 \mu\text{Eq/min}$ during the control period to $1.027 \mu\text{Eq/min}$ during the experimental period, FENa from 0.62% to 0.68% and GFR from 1.226 ml/min to 1.267 ml/min in the rat bioassay. The increment of UNaV, FENa and GFR are respectively of 13.2% , 9.67% and 3.34% .

By contrast when a fraction from an expanded rat is injected, UNaV increased from a control value of $0.7 \mu\text{Eq/min}$ to $1.871 \mu\text{Eq/min}$, FENa from 0.59% to 2.38% , and GFR decreased from 0.984 to 0.686 ml/min . Increment of UNaV, FENa and GFR were respectively of 165% , 289% , and -30.8% . FENa and UNaV increased immediately after the injection of the expanded rat fraction in the experimental period, with a peak in the second experimental period. Then, FENa decreased progressively.

The results of the bioassay measuring the activity of the natriuretic factor obtained from the renal tissue of each rat are presented in Tables IV and V. Changes in GFR during all the assays are small and inconsistent. Increment in UNaV did not change significantly when fractions from the groups of the first series were tested : group A averaged $-7.35 \pm 20.8\%$, group B $23.88 \pm 44.2\%$, and group C $3.42 \pm 13.1\%$. The mean of FENai increased in a parallel direction to the basal salt intake : it increased from $-0.45 \pm 20.46\%$ in group A to $9.2 \pm 32.66\%$ in group B, and to $29.98 \pm 17.7\%$ in group C. Statistical increase of FENai between control and experimental periods ($p < 0.0025$) was noted for the latter group (paired "t" test). However no statistical differences were calculated between the three groups.

TABLE IV
Effect of the Kidney Fraction of Non-Expanded Rats
on GFR, UNaV and FENa in the Assay

RAT	Control periods			Experimental periods			Increment of		
No.	GFR ml/min	UNaV μEq/min	FENa %	GFR ml/min	UNaV μEq/min	FENa %	GFR %	UNaV %	FENa %
G R O U P A									
1	1.506	0.489	0.31	1.171	0.152	0.13	-22.4	-68.91	-58.0
2	1.957	0.89	0.37	1.333	0.11	0.064	-29.8	-87.64	-82.7
3	0.94	1.72	1.5	0.605	0.591	0.8	-35.6	-65.63	-46.6
4	0.87	2.57	2.36	0.921	2.901	2.58	5.86	12.87	9.32
5	1.526	0.204	0.11	1.895	0.233	0.099	24.18	14.21	-10.0
6	1.838	2.92	1.327	1.546	2.28	1.367	-15.88	-21.91	3.03
7	1.109	0.903	0.62	0.988	1.47	1.12	-10.9	62.79	80.6
8	1.927	2.02	0.85	1.497	2.99	1.59	-22.3	48.0	87.0
9	0.615	0.029	0.036	1.001	0.059	0.065	62.7	103.4	80.5
10	1.64	1.21	0.59	1.253	0.354	0.19	-23.59	-70.74	-67.7
mean	1.39	1.29	0.807	1.225	1.114	0.8	- 6.77	- 7.35	- 0.45
± SE	0.15	0.31	0.23	0.11	0.38	0.27	9.52	20.8	20.46
p							NS	NS	NS
G R O U P B									
1	0.82	2.42	1.85	0.701	0.903	0.987	-14.5	-62.68	-48.2
2	2.23	0.062	0.021	1.831	0.084	0.035	-17.8	35.4	66.6
3	2.25	0.197	0.098	1.88	0.727	0.27	-16.4	269.0	175.5
4	1.053	0.14	0.09	0.869	0.115	0.106	-17.47	-17.8	17.7
5	0.72	2.26	2.145	0.572	0.639	0.81	-20.5	-71.7	-62.2
6	0.513	0.697	1.17	1.289	1.018	0.729	151.2	46.0	-37.6
7	0.692	2.44	2.85	1.08	1.683	1.497	56.0	-31.0	-47.4
mean	1.18	1.17	1.17	1.174	0.74	0.63	17.21	23.88	9.2
± SE	0.28	0.43	0.43	0.19	0.21	0.20	24.61	44.2	32.66
p							NS	NS	NS
G R O U P C									
1	2.53	0.295	0.095	1.695	0.417	0.19	-33.0	41.3	100.0
2	2.374	0.996	0.31	2.253	1.011	0.34	- 5.1	0.7	9.67
3	1.388	0.554	0.37	1.238	0.568	0.398	-10.8	2.52	7.56
4	1.226	0.907	0.62	1.267	1.027	0.68	3.34	13.2	9.67
5	1.73	0.539	0.26	0.889	0.32	0.32	-48.6	-40.6	23.0
mean	1.84	0.66	0.33	1.47	0.67	0.385	-18.83	3.42	29.98
± SE	0.26	0.13	0.08	0.23	0.15	0.08	9.56	13.1	17.7
p							NS	NS	<0.0025

The mean values for GFR, UNaV and FENa for the clearance periods obtained over the six experimental periods after infusion of the fractions were compared with the mean values obtained during the four control periods. The activity of the fractions is defined by the increment of the three parameters during the experiment. Groups of rats are defined in Table I.

TABLE V
Effect of the Kidney Fraction of Expanded Rats
on GFR, UNaV and FENa in the Assay

RAT No.	Control periods			Experimental periods			Increment of		
	GFR ml/min	UNaV μ Eq/min	FENa %	GFR ml/min	UNaV μ Eq/min	FENa %	GFR %	UNaV %	FENa %
G R O U P A _E									
1	1.192	0.532	0.36	1.236	1.03	0.726	3.69	93.6	101.65
2	1.024	0.508	0.44	0.55	0.784	1.286	-45.0	54.33	192.27
3	1.14	0.424	0.324	1.062	0.458	0.369	-6.84	7.42	13.88
4	1.815	0.105	0.051	1.514	0.318	0.176	-16.58	202.85	245.0
5	0.674	1.73	1.98	0.908	3.12	3.27	34.71	80.34	65.15
mean	1.17	0.66	0.63	1.055	1.14	1.165	-6.16	87.7	123.59
$\pm$ SE	0.18	0.28	0.34	0.16	0.51	0.56	13.13	32.3	42.06
P							NS	<0.05	<0.05
G R O U P B _E									
1	1.722	0.114	0.04	1.449	0.432	0.258	-15.85	278.94	475.0
2	1.843	0.4	0.216	1.718	2.669	1.158	-6.78	567.25	431.1
3	1.875	0.755	0.357	1.14	1.794	1.245	-39.21	137.6	248.7
4	2.05	0.478	0.212	1.117	1.25	1.003	-45.51	161.5	373.1
5	0.977	2.79	2.7	1.131	6.098	5.07	15.76	118.56	85.4
6	0.984	0.706	0.59	0.686	1.871	2.308	-30.28	165.0	289.8
mean	1.58	0.87	0.685	1.206	2.35	1.8	-20.31	238.14	317.18
$\pm$ SE	0.19	0.39	0.41	0.14	0.81	0.7	9.3	69.67	57.8
P							<0.05	<0.0125	<0.01
G R O U P C _E									
1	1.437	0.054	0.026	1.107	0.142	0.092	-22.96	163.5	253.8
2	1.872	0.207	0.081	1.333	0.645	0.361	-28.79	211.5	351.25
3	2.075	0.625	0.21	1.97	1.81	0.686	-5.06	189.6	226.66
4	0.792	0.1	0.1	0.543	0.6	1.01	-31.43	494.3	868.75
mean	1.544	0.246	0.104	1.238	0.769	0.537	-22.06	264.72	425.11
$\pm$ SE	0.28	0.13	0.04	0.29	0.35	0.2	5.94	77.15	150.3
P							<0.025	<0.05	<0.05

Increment of GFR, UNaV and FENa are defined in Table IV. Groups of rats are defined in Table I.

By contrast in the second series fractions from groups A_E , B_E , and C_E showed a significant increment of UNaV in the assays with an average of $87.7 \pm 32.3\%$ ($p < 0.05$) for group A_E , $238.14 \pm 69.67\%$ for group B_E ($p < 0.0125$), $264.72 \pm 77.15\%$ in the group C_E ($p < 0.05$). The mean increment of the FENa paralleled the one of UNaV : $123.59 \pm 42.06\%$ ($p < 0.05$) for group A_E , $317.18 \pm 57.8\%$ ($p < 0.01$) for group B_E , and $425.11 \pm 152.3\%$ ($p < 0.05$) for group C_E . FENaI of the different groups is presented in Fig. 2.

When the activity of the fraction of the same paired groups (expanded and non-expanded groups) was compared, a significant difference in the UNaVi and the FENaI existed : A_E vs A $p < 0.025$ and $p < 0.01$ *for both UNaVi and FENaI respectively, B_E vs B $p < 0.01$ and $p < 0.0025$, C_E vs C $p < 0.01$ and $p < 0.0125$.

When the three expanded groups were compared the following results were obtained : B_E and C_E vs A_E showed a $p < 0.01$ for both UNaVi and FENaI.

DISCUSSION

The present study has been designed to test the hypothesis that the natriuretic factor found in chronic models in relation to the amount of sodium to be eliminated by the kidneys is also a determinant component of the control system which governs the natriuretic response to an acute saline load. Thus, the natriuretic factor has been prepared according to the same methodology as used previously when response to a chronic load of sodium was investigated (5-7).

After passage through a G-25 Sephadex column using an acetate ammonium eluate at pH 6.8 (5), the natriuretic activity of the extracts from the rat kidney used in the present investigation has been found in the same peak appearing immediately after the peak containing the sodium and other solutes which has been repeatedly demonstrated to possess the natriuretic activity when urine extracts from animals and human submitted to a chronic high salt intake have been previously studied. In addition, as depicted in fig. 1, this natriuretic material when injected into the

rat induces a natriuresis following the same profile as reported before in chronic experiments. This response is characterized by an immediate increase of the sodium excretion by the rat assay which lasts about 40-60 minutes. This kind of natriuretic response has been described by de Wardener (12) as typical for the low molecular weight natriuretic substance. Therefore, as a similar chemical procedure has been used and as a similar effect of the fraction on the assay has been observed, it could be assumed but not proven, in the absence of chemical characterization, that we are dealing in these acute experiments with the same material as the one largely demonstrated in urine from chronically expanded dogs and human (7-8). Natriuretic response to an acute load of sodium is known to depend on the basal sodium diet to which the animals are submitted before the acute experiments (15-16). Thus, it is worthwhile to establish the degree of the basal activity of the natriuretic factor which could be found in the rat kidney in relation to the salt intake. In the present investigation three different sodium intakes have been studied i.e. 0, 3 and 6 mEq/day (Table I). Assuming that the kidney is at least the receptor organ for the natriuretic factor, it should be expected to find an increasing activity of the natriuretic factor in the kidney when rats are given a larger amount of salt.

Table III shows that after fasting, the fractional sodium excretion is similar for all the groups (A, B, C), a result which is not unexpected as the ingested sodium is known to be eliminated in five hours (19). Thus, this similitude in FENa for the three groups is an argument demonstrating that a basal state has been actually reached at the time where the kidneys are removed. As Fig. 2 illustrates, there is a progressive increasing in the activity of the natriuretic factor when the material is extracted from kidneys of Group A compared to the material provided by groups B and C. However, in all the groups this activity is small and only statistically significant for group C, when FENa in the assay obtained during the control period is compared to the experimental periods (paired "t" test $p < 0.0025$). Under basal conditions, the renal tissue exhibits an activity of the natriuretic factor which parallels the amount of salt ingested by the donor as showed in Fig. 2, although the difference in natriuretic activity between the groups does not reach the level of significance. However, this relationship between the salt content

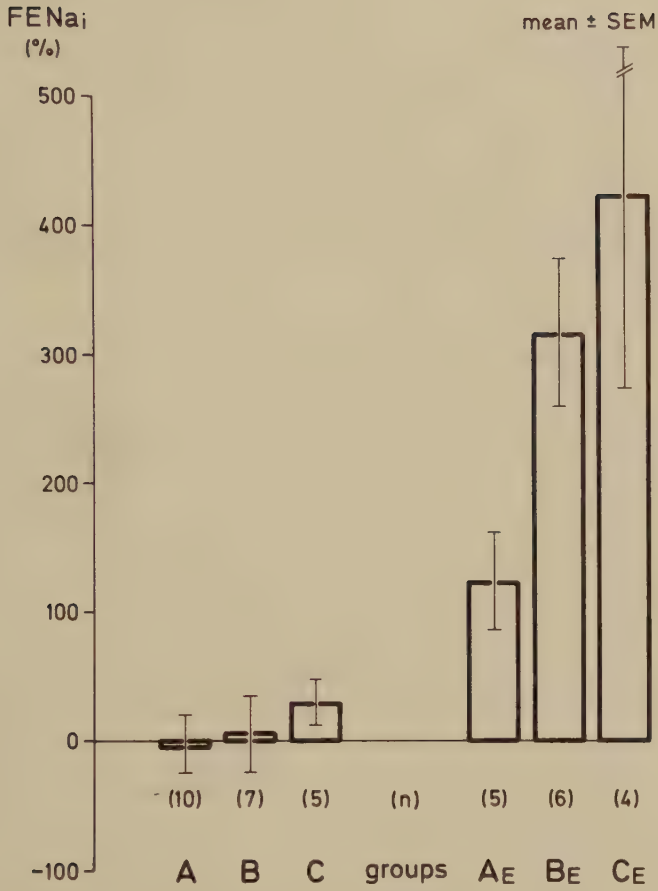


FIGURE 2. Activity of the natriuretic factor extracted from the total renal tissue expressed by FENai between the different experimental groups. Groups are defined in Table I.

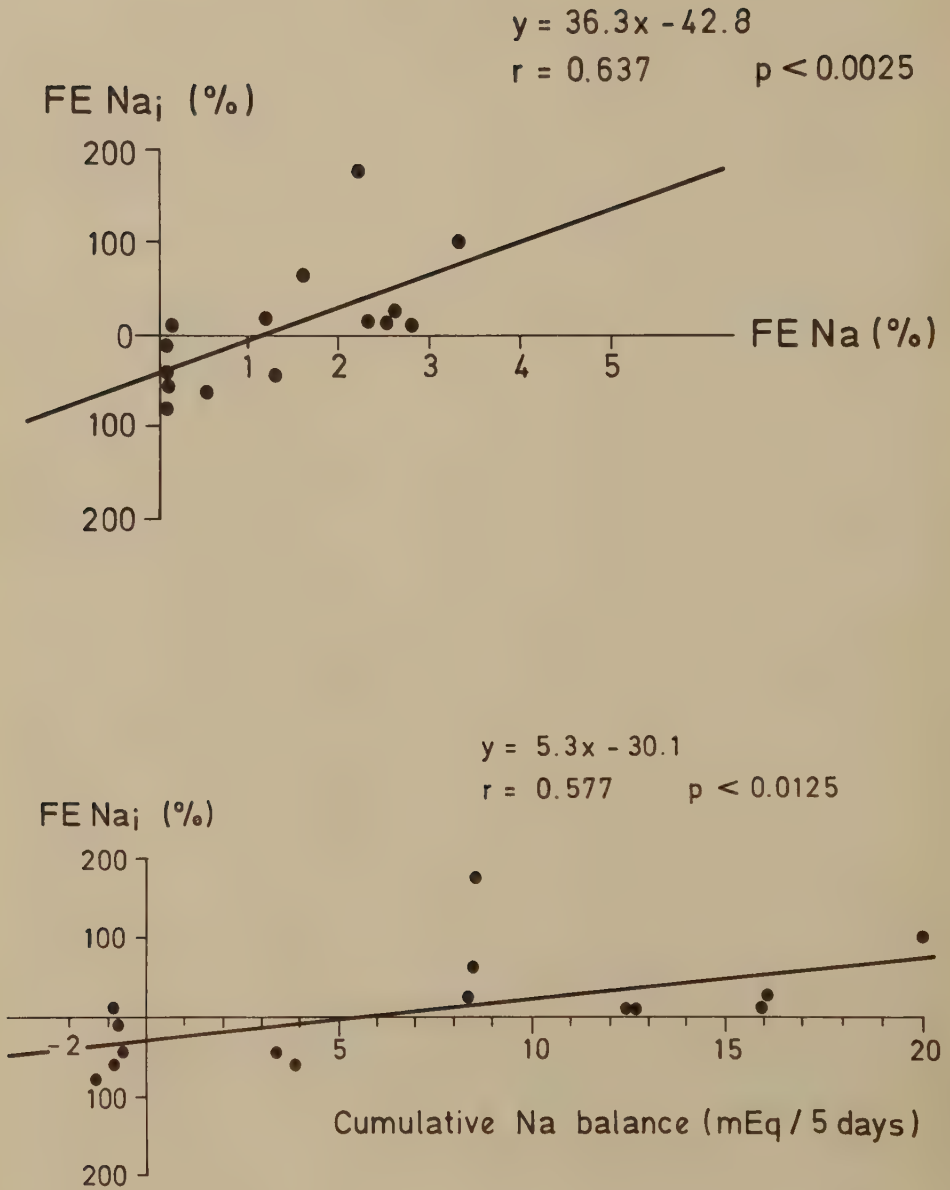


FIGURE 3. Relationship between the activity of the natriuretic fraction extracted from kidneys of the donor rats expressed by FENa_i (change of FENa in per cent during the assay) and their FENa value calculated on the basis of a 24-hour urine collection on the tenth day in metabolic cage (upper panel) or their 5-day cumulative sodium balance (sodium intake -UNaV, mEq/5 days, lower panel).

of the diet and the natriuretic activity found in the kidney is strengthened by the fact that it exists a close correlation ($r = 0.64$ $p < 0.0025$) between the FENa observed over a 24-hour urine collection calculated on the tenth day in metabolic cage and the activity of the natriuretic factor obtained from the same animal. There is also a similar correlation ($r = 0.58$ $p < 0.0125$) between the 5-day cumulative sodium balance and the activity of the natriuretic factor as shown in Fig. 3. Now let us consider the effects of acute saline expansion on the activity of natriuretic factor found in the kidney tissue.

It has been proven in rats as well as in dogs by micropuncture studies that acute saline expansion results in an inhibition of the sodium reabsorption by the end of the proximal tubule promoting an increase of the distal delivery (20). In these protocols, a marked final natriuresis is observed. By contrast, when expansion of a similar degree is reached by perfusion of albumine, although the same degree of inhibition of the sodium reabsorption is observed by the end of the proximal tubule, no significant final natriuresis occurs (21-22). The difference between the two protocols is the compartments concerned by the kind of expansion, the former involves the extracellular compartment while the latter is restricted to the vascular compartment (21). Clearly with an expansion of the extracellular compartment, changes in sodium transport take place in the distal segment of the nephron, an area where no physico-chemical factors are known to play a role in the sodium handling by the kidney. Therefore, a determinant factor for the final natriuresis found after acute saline expansion must inhibit the sodium transport by the distal segment of the nephron. Natriuretic factor prepared similarly to the one used in the present work and possessing the same biological effects when injected into the rat is likely to act on distal segment. For long, Bourgoignie showed that this natriuretic factor inhibits the SCC when applied in an anurion membrane preparation (5), a structure which is considered as having similar properties, as far as the sodium transport is concerned as the distal segment of the nephron. More recently, using a rabbit isolated collecting duct preparation, Fine has directly demonstrated that the natriuretic factor obtained from urine of uremic patients is active on that segment (23).

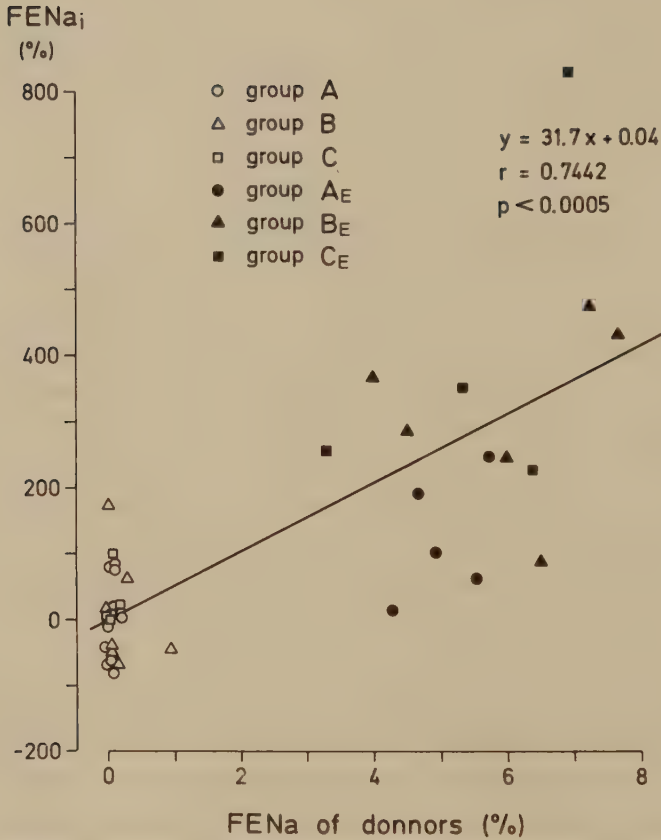


FIGURE 4. Relationship between the FENa calculated immediately before the removing of the kidneys and the activity found in the fraction extracted from their corresponding kidneys. FENa_i relates to the change of FENa observed in the assay and FENa to the value observed in the donor rats.

Thus, if one accepts that the natriuretic factor found in the present study is the same as the one previously demonstrated from urine of men and dogs, it would be a good candidate as responsible for the natriuresis observed in the rat after acute expansion.

As shown in Fig. 2, all the groups of rats respond to the acute load of saline by exhibiting a highly significant activity of their natriuretic factor found in their kidneys. This activity is in relationship with the fractional excretion of sodium observed in the rat after expansion and just before the kidneys were removed ($r = 0.46$ $p < 0.05$). When rats of all groups are considered altogether, a close correlation exists ($r = 0.74$ $p < 0.0005$), as shown in Fig. 4. Of interest is the fact that the activity of the natriuretic factor is significantly greater in the animals of groups B_E and C_E compared to A_E ($p < 0.01$). This finding is in agreement with the observation that the fractional sodium excretion obtained in donor rats from groups B_E and C_E is significantly greater than the one from animals of group A_E ($p < 0.0125$). This fact emphasizes the importance of the basic sodium diet on the response to an acute load of salt.

In conclusion, basal activity could be demonstrated in the rat kidney in relation with the sodium content of the diet. After acute saline expansion, the natriuretic factor activity increases significantly in a parallel direction to the importance of the natriuresis observed in the donor rats, the size of which depends on the previous basal sodium intakes. Thus, natriuretic factor must be considered as a major component of the regulation of the sodium excretion by the kidney in acute situation as well as in chronic state.

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